FIGURE S1

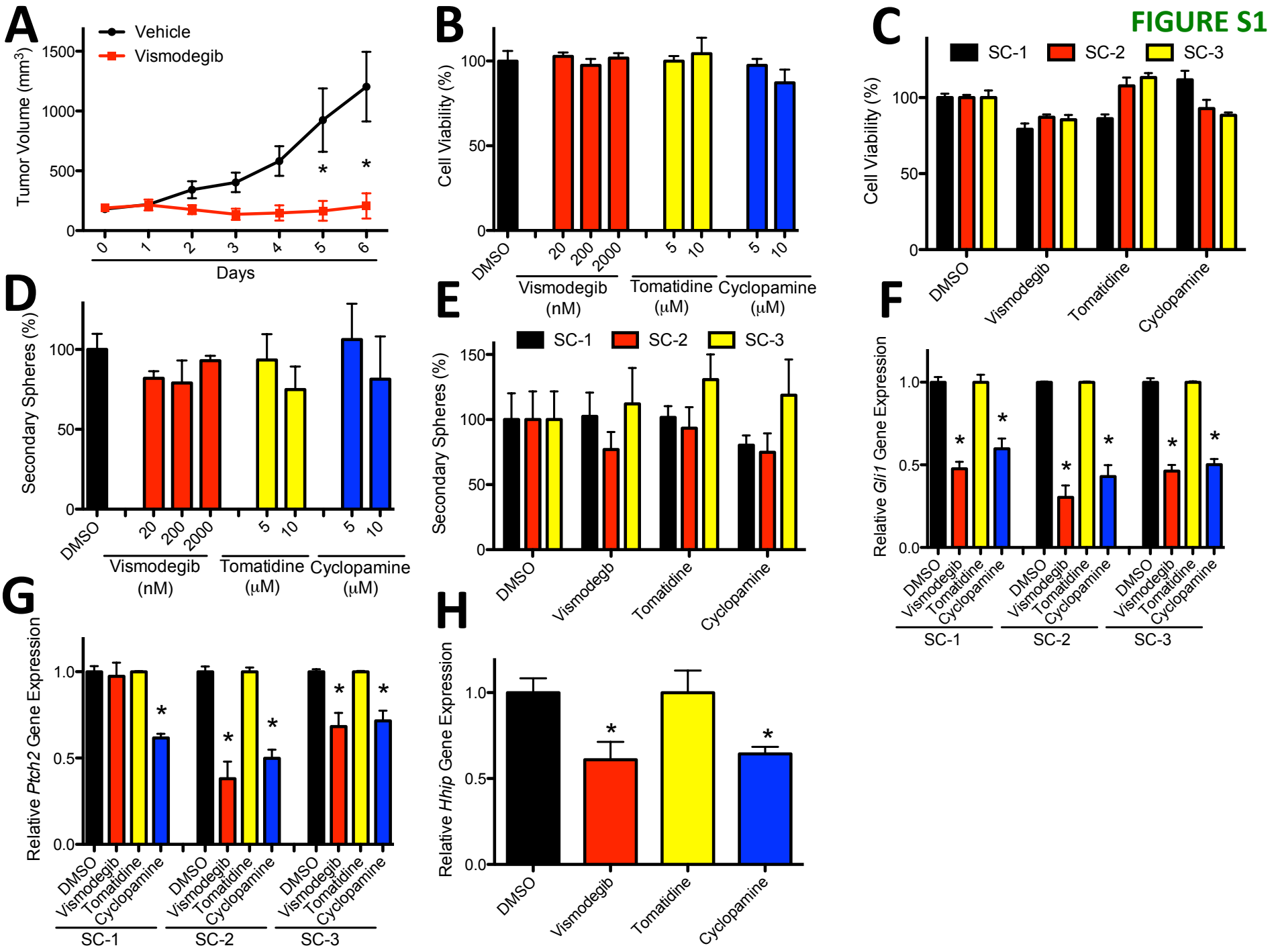
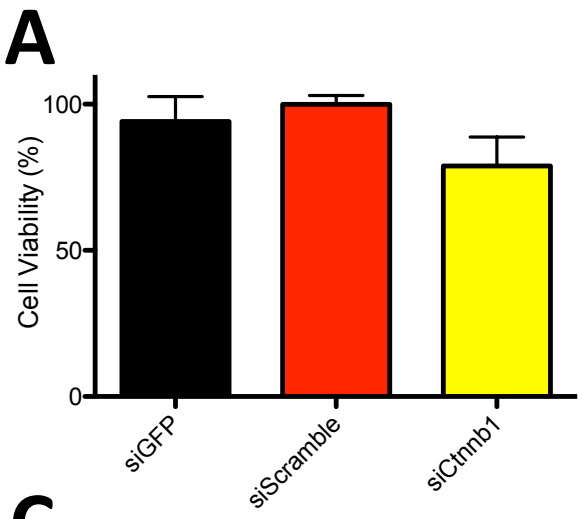
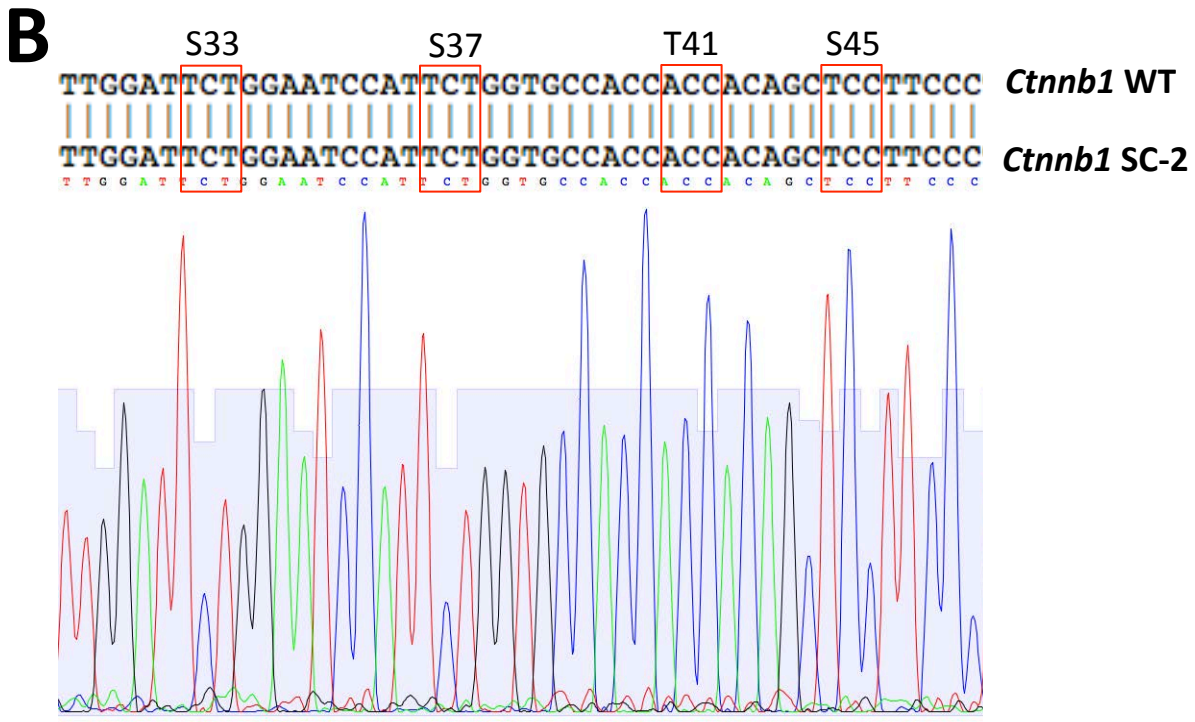

A
Vehicle
Vismodegib
Tumor Volume (mm3)
1500
1000
500
0
0 1 2 3 4 5 6
Days
*
*
B
Cell Viability (%)
100
50
0
DMSO
20 200 2000
5 10
5 10
Vismodegib (nM)
Tomatidine (μM)
Cyclopamine (μM)
C
SC-1
SC-2
SC-3
Cell Viability (%)
100
50
0
DMSO
Vismodegib
Tomatidine
Cyclopamine
D
Secondary Spheres (%)
100
50
0
DMSO
20 200 2000
5 10
5 10
Vismodegib (nM)
Tomatidine (μM)
Cyclopamine (μM)
E
SC-1
SC-2
SC-3
Secondary Spheres (%)
150
100
50
0
DMSO
Vismodegb
Tomatidine
Cyclopamine
F
Relative Gli1 Gene Expression
1.0
0.5
0.0
DMSO
Vismodegib
Tomatidine
Cyclopamine
SC-1
SC-2
SC-3
G
Relative Ptch2 Gene Expression
1.0
0.5
0.0
DMSO
Vismodegib
Tomatidine
Cyclopamine
SC-1
SC-2
SC-3
H
Relative Hhip Gene Expression
1.0
0.5
0.0
DMSO
Vismodegib
Tomatidine
Cyclopamine

Figure S1: Medulloblastoma sphere cultures are insensitive to SMOOTHENED inhibitors. (**A**) *Ptch1* driven, *Trp53* mutant MB tissue, which had never been cultured *ex vivo*, was subcutaneously implanted into the flanks of immunocompromised mice. Mice were treated daily with 50 mg/kg of vismodegib, or vehicle control, for indicated days. (**B**) SC-2 was incubated with the indicated concentrations of SMO inhibitor for 3 days, prior to determining cell viability using a MTT reduction assay. (**C**) The viability of the indicated SCs was determined following 3 days incubation with DMSO, 200 nM vismodegib, 10 µM tomatidine or 10 µM cyclopamine. (**D**) The ability SC-2 to form secondary spheres was determined following incubation (24 hours) with the indicated concentrations of inhibitor. (**E**) The ability of the indicated SC to form secondary spheres was determined following incubation (24 hours) with DMSO, 200 nM vismodegib, 10 µM tomatidine or 10 µM cyclopamine. Data was normalized to DMSO control. (**F**) The expression of the SHH target gene *Gli1* was determined in the indicated SCs treated with DMSO, 200 nM vismodegib, 10 µM tomatidine, or 10 µM cyclopamine (24 hours). (**G**) The expression of the SHH target gene *Ptch2* was determined in the indicated SCs treated with DMSO, 200 nM vismodegib, 10 µM tomatidine, or 10 µM cyclopamine (24 hours). (**H**) The expression of the SHH target gene *Hhip* was determined in SC-2 treated with DMSO, 200 nM vismodegib, 10 µM tomatidine, or 10 µM cyclopamine (24 hours). Vismodegib was normalized to DMSO, while cyclopamine was to tomatidine unless otherwise indicated.





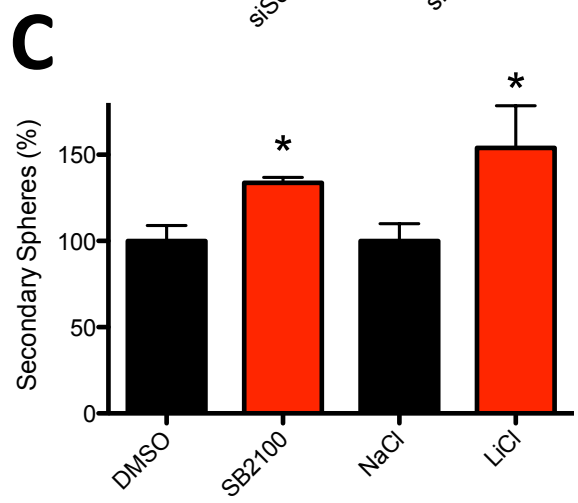


Figure S2: The viability of medulloblastoma sphere cultures is not WNT-dependent. (**A**) SC-2 was transfected with the indicated siRNA smart pools and cell viability determined 5 days later, using a MTT reduction assay. Results were normalized to those from the siScramble control. (**B**) The exon 3 of the β-Catenin (Ctnnb1) gene was sequenced and aligned to wild type (WT) sequence. Mutational hotspot for SC-2 is shown. (**C**) The ability of SC-2 to form secondary spheres was determined following incubation (24 hours) with the small molecule WNT agonists SB2100 (10 µM) or lithium chloride (LiCl) (10 mM). SB2100 data was normalized to a DMSO control, while LiCl data was normalized to a 10 mM sodium chloride (NaCl) control.

Figure S3

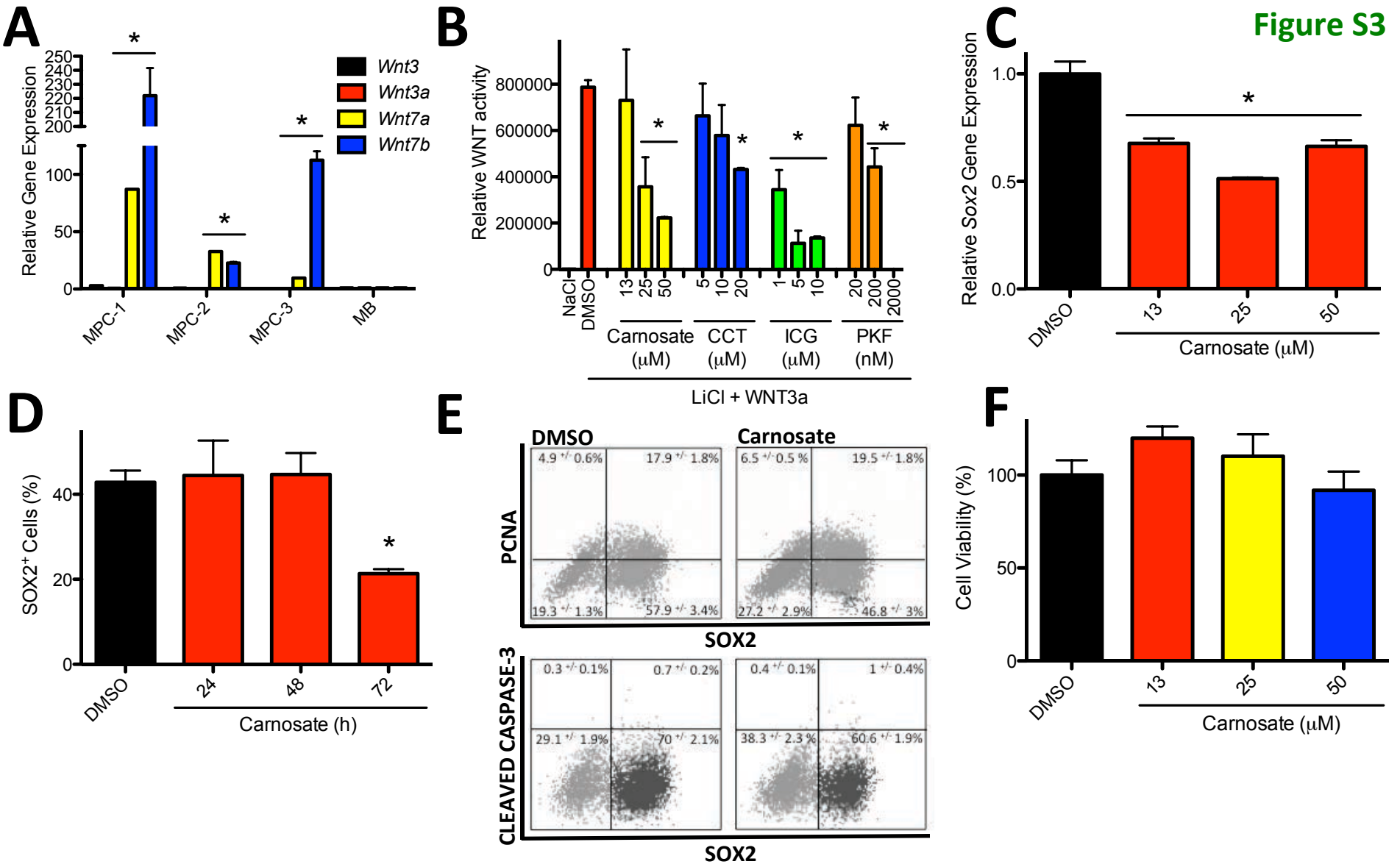

A
Relative Gene Expression
Wnt3
Wnt3a
Wnt7a
Wnt7b
MPC-1
MPC-2
MPC-3
MB
B
Relative WNT activity
NaCl
DMSO
Carnosate (μM)
CCT (μM)
ICG (μM)
PKF (nM)
LiCl + WNT3a
C
Relative Sox2 Gene Expression
DMSO
Carnosate (μM)
D
SOX2+ Cells (%)
DMSO
24
48
72
Carnosate (h)
E
DMSO
Carnosate
PCNA
SOX2
CLEAVED CASPASE-3
SOX2
F
Cell Viability (%)
DMSO
Carnosate (μM)

Figure S3: Small-molecule WNT inhibitors attenuate the self-renewal of medulloblastoma sphere cultures. (**A**) The expression of *Wnt* family members in the indicated SC was analyzed and compared to the average expression of 5 independent MB samples. (**B**) Validation of various small-molecule WNT inhibitors was determined using a TCF/LEF driven luciferase reporter cell line (293STF). These cells were treated with WNT protein and LiCl for 24 hours, and then exposed to the indicated concentrations of TCF/β-CATENIN inhibitors for an additional 24 hours. Results were then normalized to that of the DMSO/NaCl control. (**C**) The relative expression of the MPC biomarker *Sox2* was determined following incubation of SC-2 (24 hours) with the indicated concentrations of carnosate. (**D**) Quantitation of $SOX2^{+}$ cells was determined, by FACS analysis, in SC-2 treated with carnosate (25 μM) for the indicated times. (**E**) SC-2 was treated for 3 days with carnosate (25 μM) before be stained for SOX2 and PCNA, or SOX2 and CLEAVED CASPASE-3. Positively stained cells were quantitated by FACS analyses, using the indicated cutoffs. (**F**). SC-2 was incubated for 6 days with indicated concentrations of carnosate prior to determining cell viability using a MTT reduction assay. Data was normalized to DMSO unless otherwise indicated.

A

FIGURE S4

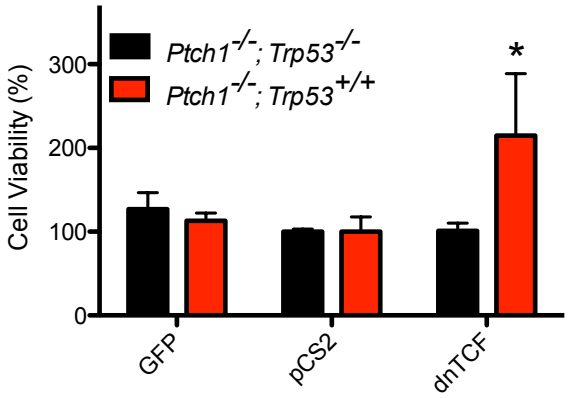


Figure S4: MPC enriched cultures expressing wild type *Trp53* are not WNT-dependent. (**A**) SC-2 was transfected with plasmids expressing *dnTCF3*, a control plasmid (*pCS2*), or *GFP*, and cell viability determined 5 days later using a MTT reduction assay. Data was normalized to *pCS2* control.

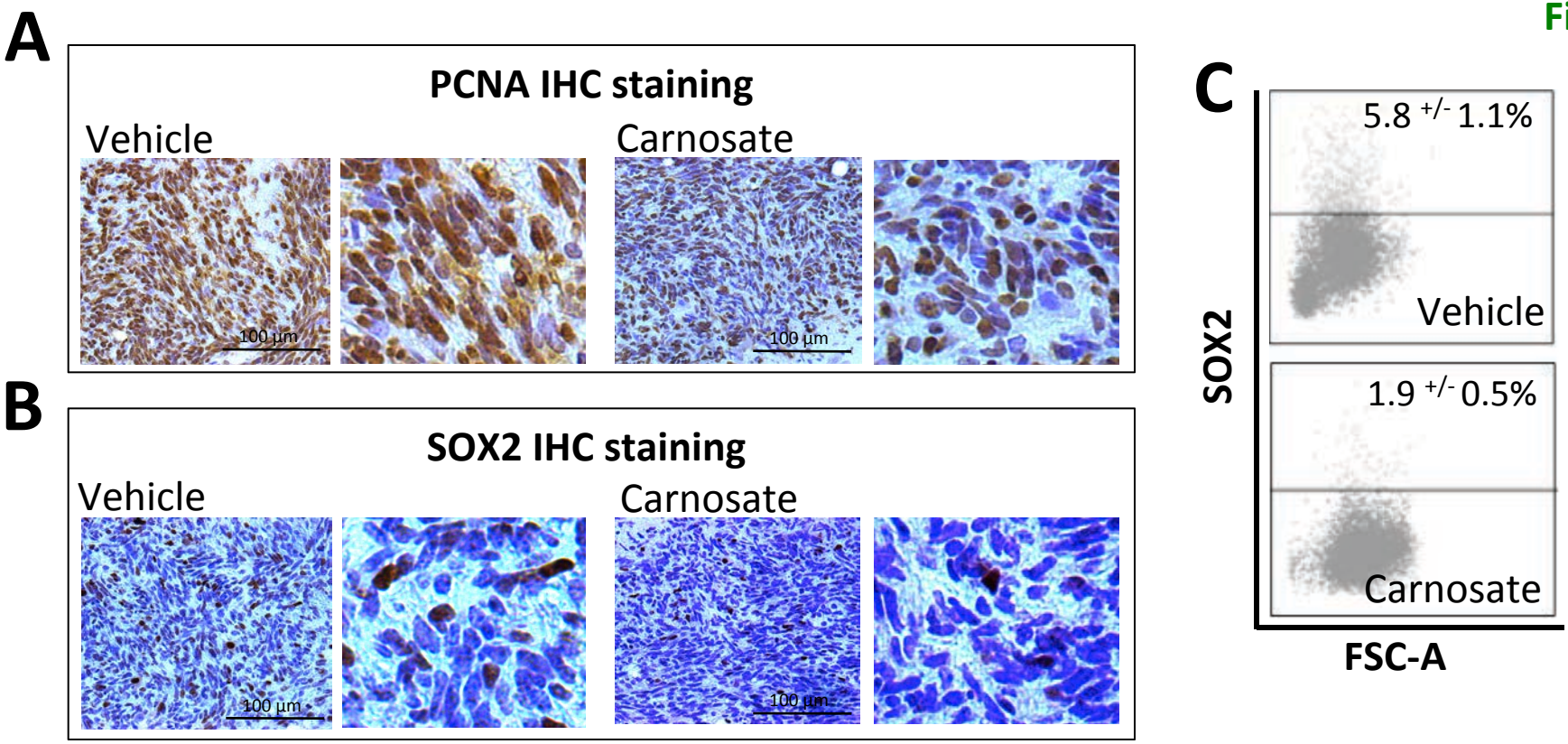


Figure S5: Medulloblastoma growth and propagation is WNT-dependent. MB tissue, which had never been cultured *ex vivo*, was orthotopically implanted into the cerebellum of immunocompromised mice, and these mice treated with carnosate or vehicle. Twenty days after treatment, or upon developing MB symptoms, the mice were sacrificed and their brains harvested and analyzed as described in Figure 5. Representative images of (**A**) PCNA or (**B**) SOX2 immunostaining are shown here. Both low and higher magnification images are shown for each biomarker. (**C**) The enrichment of $SOX2^{+}$ cells in residual flank tumors was determined by FACS analysis, using the indicated parameters.

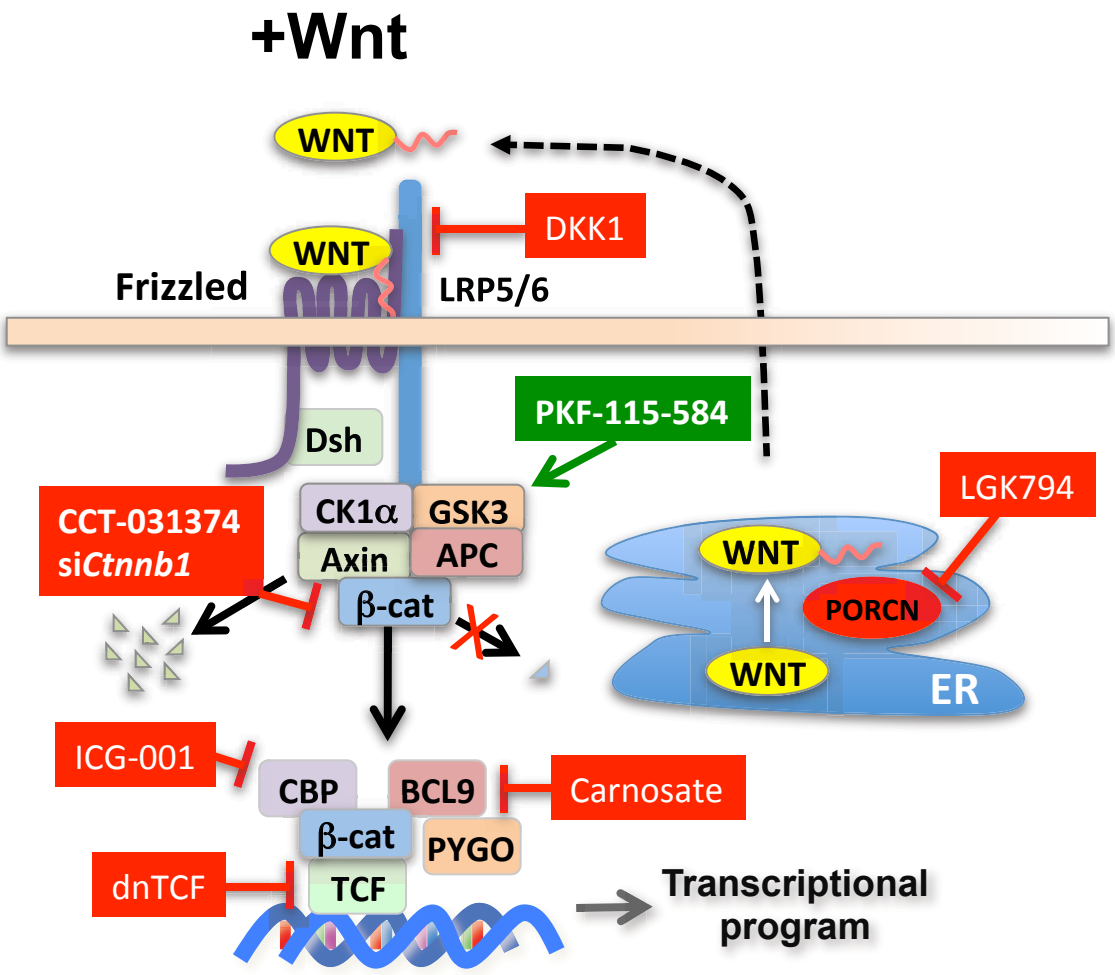


Figure S6: Mechanism of action of various inhibitors of WNT signaling. See text for details.